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Differential response of maize catalases to abscisic acid: Vp1 transcriptional activator is not required for abscisic acid-regulated *Cat1* expression

(oxidative stress/viviparous mutants/isozymes/embryogenesis/gene expression)

JOHN D. WILLIAMSON AND JOHN G. SCANDALIOS*

Department of Genetics, Box 7614, North Carolina State University, Raleigh, NC 27695-7614

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ABSTRACT **In this paper we describe the distinctive responses of the maize catalases to the plant growth regulator abscisic acid (ABA). We analyzed RNA and enzyme accumulation in excised maize embryos and found that each catalase responded differently to exogenously applied ABA. Levels of *Cat1* transcript and enzyme activity rapidly increased. In contrast, levels of *Cat2* transcript and protein decreased, while *Cat3* transcript levels were not affected. In developing kernels of the ABA-deficient/biosynthetic viviparous mutant *vp5*, lower levels of *Cat1* RNA correlated with lower endogenous ABA levels when compared to measured levels in comparably aged wild-type siblings from the same ear. The maize *vp1* mutant line is morphologically insensitive to normal endogenous levels of ABA. Analysis of the response of *Cat1* to exogenously applied ABA in mutant and wild-type *vp1* sibling embryos suggests that, unlike other ABA-responsive genes analyzed to date, the *Vp1* gene product is not essential for the ABA-mediated regulation of *Cat1*. The significance of these responses to ABA in defining the roles of the various CATs in maize is discussed.**

Catalase (CAT; H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) is a tetrameric, heme-containing enzyme that catalyzes the dismutation of H_2O_2 into H_2O and O_2. In maize (*Zea mays* L.) three unlinked CAT structural genes, *Cat1*, *Cat2*, and *Cat3*, encode three biochemically distinct isozyme subunits, CAT-1, CAT-2, and CAT-3, of ≈60 kDa, which are structurally similar to CATs found in other organisms (1–3). The differential spatial and temporal expression of these isozymes in maize has been described (3–6). CAT-1 is the only CAT isozyme that accumulates to detectable levels in mature pollen, in milky endosperm, and in the scutellum during early kernel development. The CAT-2 isozyme begins to accumulate in the aleurone and scutella 20–22 days postpollination. Upon inhibition, levels of CAT-2 increase, with the CAT-2 accumulation profile paralleling that of the glyoxysomes, the primary intracellular location of this isozyme (7).

Because the pattern of CAT protein accumulation in maize scutella shifts during developmental stages characterized by changes in endogenous phytohormone levels (8), we conducted analyses to assess the effect of abscisic acid (ABA) on the expression of catalases during maize embryogenesis. We examined the dose–response and kinetics of *Cat* transcript accumulation in excised maize embryos in response to exogenously applied ABA. In addition, we used two maize viviparous (*vp*) mutant lines to assess the effect of changes in normal endogenous ABA levels on *Cat* transcript accumulation *in planta* and to determine whether or not the ABA-regulated transcriptional activator Vp1 mediated this response. The maize *vp* mutant lines utilized make either lowered amounts of ABA (*vp5*) or are phenotypically insensitive to normal endogenous levels of ABA (*vp1*) due to the absence of the *Vp1* gene product (9–11). Tracer competition ELISA was used to correlate *in vivo* changes in ABA levels with *Cat* transcript levels during embryogenesis in these two lines. Our results show that the three CATs are differentially regulated in the presence of ABA and that this regulation is apparently not mediated by *Vp1*.

MATERIALS AND METHODS

Plants and Plant Growth Conditions. The maize inbred lines W64A, M1A4 (*Vp5/vp5*, *Y/y*), and 326B (*Vp1/vp1R*, A_1A_2, C_1C_2) were used in these studies. W64A and M1A4 are maintained in this laboratory; line 326B was obtained from the Maize Stock Center (University of Illinois, Urbana). The maize *vp* mutant lines utilized make either lowered amounts of ABA (line M1A4; *vp5*) or are morphologically insensitive to normal endogenous levels of ABA (line 326B; *vp1*) (9, 10), resulting in precocious germination in the ear. Since precocious germination occurs fairly late in development, viviparous kernels were distinguished from wild type at earlier stages on the basis of endosperm and embryo color. The *vp5* mutation in line M1A4 interrupts ABA biosynthesis early in the biosynthetic pathway (10). Homozygous recessive kernels (*vp5/vp5*), therefore, lack carotenoids, and the resulting white endosperm is easily distinguished from the yellow wild-type phenotype. Mutant embryos are also white, allowing us to distinguish viviparous kernels from the nonviviparous, white endosperm mutation (*y/y*) that has yellow embryos. In line 326B, the *vp1* mutant results in greatly reduced production of an ABA-induced transcription factor (11). In addition to the viviparous phenotype, this pleiotropic mutation results in decreased anthocyanin biosynthesis in the kernel. The homozygous mutant (*vp1/vp1*) is, therefore, characterized by yellow kernels in contrast to the wild-type red kernels. Since both of these recessive mutations are lethal in the homozygous state, they are maintained as heterozygotes.

Field-grown ears were harvested between 8 and 9 a.m., and whole embryos were manually excised between noon and 3 p.m. of the same day. Excised embryos were incubated on plates consisting of growth medium (GM) (10) or GM supplemented with filter-sterilized ABA. Excised embryos were incubated in the dark at 25°C for the indicated period of time. At the conclusion of treatment, scutella (embryo minus axis) were manually isolated, frozen in liquid nitrogen, and stored at −70°C.

Preparation of Dose–Response Plates. All ABA supplements were prepared by appropriate dilutions (using 0.1 M KOH) of a 10^{-2} M stock of a racemic mixture of ABA (Sigma

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Abbreviations: ABA, abscisic acid; CAT, catalase; dpp, days postpollination; GM, growth medium.

*To whom reprint requests should be addressed.

no. A-1049) dissolved in 0.1 M KOH. Because only the + or (*R*) enantiomer of ABA is active, effective concentrations of ABA are one-half the indicated total concentrations. In embryo dose–response studies, GM was supplemented with either KOH alone or the indicated dilution of the racemic ABA stock. Based on the results of dose–response studies of accumulation of *Cat1* and early methionine-labeled polypeptide (E_m) transcripts, +ABA plates for all other analyses contained 10^{-4} M racemic ABA [5×10^{-5} M (R)-ABA]. For kinetic studies, embryos were incubated in the dark for 0–24 hr, and samples were harvested at specified intervals. All other treatments were for 24 hr unless otherwise indicated.

Zymogram Analyses. Electrophoresis using a Tris citrate buffer system was used for analyses of CAT activity in equal protein samples as described (12). Protein was determined by the method of Lowry *et al.* (13).

RNA Analyses. Total RNA was isolated from tissue samples by a cold phenol extraction method (14). For Northern blot analysis of transcript accumulation, total RNA (20 μg) from each sample was separated on denaturing 1.2% agarose gels and transferred to nitrocellulose (15). The resulting blots were hybridized with ^{32}P-labeled, sequence-specific probes prepared from cDNAs for *Cat1*, *Cat2*, and *Cat3* (16, 17). After these analyses had been performed, probes were removed from the filters by repeated washes in boiling 0.1× standard saline citrate/0.1% SDS. Filters were reprobed first with ^{32}P-labeled insert DNA from clone p1015 (18) containing the entire coding sequence of the ABA-regulated E_m polypeptide and subsequently with a fragment from clone pHA2 containing an 18S ribosomal sequence (19). Because blots probed with ribosomal sequences are easily overexposed, a blot containing a dilution series of total RNA from untreated maize scutella was included to ensure exposure times resulting in a linear signal response.

ABA Determination. Endogenous ABA levels were determined by tracer competition immunoassays (20). For ABA extractions, scutella were frozen in liquid N_2, lyophilized, and then ground to a fine powder in liquid N_2. The lyophilized powder (50 mg) was extracted in 1.2 ml of cold methanol containing butylated hydroxytoluene (100 μg/ml) and citric acid monohydrate (0.5 mg/ml) (20, 21). The resulting supernatant was dried in a Speed-Vac concentrator (Savant), resuspended in 100 μl of methanol, and diluted to 1 ml with distilled H_2O. Assays were performed by using the Phytodetek-ABA kit (IDETEK, San Bruno, CA). All samples were assayed in duplicate at two different dilutions (5 and 2.5 mg dry weight equivalents), and results from three independent replicates were averaged.

RESULTS

Differential CAT Isozyme Accumulation in the Scutella of Excised Maize Embryos in Response to Exogenous ABA. Initial investigations to assess the effect of ABA on the accumulation of the various CATs used a plate assay (10). Embryos excised from developing kernels of plants of the normal inbred maize line W64A at 18–21 days postpollination (dpp) germinate precociously when incubated on simple organ culture GM. However, when excised embryos are incubated on GM supplemented with ABA, they do not germinate, but continue to mature (Fig. 1*A*).

Proteins were extracted from the scutella of embryos incubated for 3 days in the presence or absence of ABA, and relative levels of the various CAT isozymes in equal protein samples were estimated by zymogram analysis. Protein extracts from liquid endosperm, etiolated coleoptile, and 3-day postimbibition scutella were included as isozyme migration markers. These results (Fig. 1*B*) show that both CAT-1 and CAT-3 (but not CAT-2) accumulated in embryos incubated on GM supplemented with ABA (+ABA). In contrast, proteins isolated from scutella of embryos incubated on GM alone (−ABA) showed the high level of the CAT-2 isozyme accumulation characteristic of early germination.

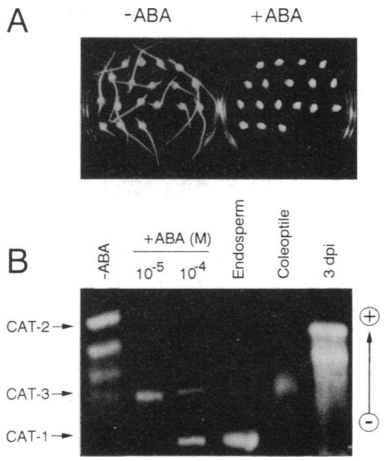

FIG. 1. Differential accumulation of CAT isozyme activities in the presence and absence of exogenous ABA. (*A*) Embryos were excised from kernels of the maize inbred line W64A at 18–21 dpp and incubated for 3 days in the dark on GM (−ABA) or GM supplemented with 10^{-4} M ABA (+ABA). (*B*) Total scutellar proteins were extracted from the above, as well as from embryos incubated in the presence of 10^{-5} M ABA and from 3 day postimbibition (3 dpi) scutella, and subjected to CAT zymogram analysis. Extracts from developing endosperm and etiolated coleoptile were included as CAT-1 and CAT-3 homotetramer markers, respectively. Migration on gels is anodal. Positions of the CAT homotetramers are indicated; remaining bands are CAT-1/CAT-2 heterotetramers.

These observations suggest that accumulation of the CAT-2 isozyme is down-regulated in the scutellum of developing maize embryos in the presence of ABA. Conversely, CAT-1 and/or CAT-3 accumulation might be up-regulated by ABA.

Accumulation of the *Cat* Transcripts in the Presence and Absence of Exogenously Applied ABA. In light of these results, we conducted a parallel time course study to assess the accumulation of *Cat* transcripts in both the presence and absence of ABA. Developing kernels were harvested at 20 dpp from plants (normal inbred line W64A), and whole embryos were manually excised. Embryos were incubated for 0, 4, and 48 hr at 25°C in the dark on GM or GM supplemented with ABA. At the conclusion of the treatment, scutella were separated from the embryo axis, and the levels of the various *Cat* transcripts in the scutella were determined by Northern blot analysis of total RNA. Accumulation of both the ABA-regulated E_m transcript and the *Cat1* transcript increased (Fig. 2) in the presence of ABA [compare the "+" and "−" (ABA) 48-hr time points]. Conversely, *Cat2* levels were lower. Levels of *Cat3* transcript were the same in the presence and absence of ABA; they first decreased and then increased over time. Our initial hypothesis is that this is a manifestation of the circadian-regulated diurnal variation in *Cat3* transcript levels seen in other tissues of the maize plant (ref. 22; M. Abler and J.G.S., unpublished results).

Dose–Response and Kinetics of Accumulation of *Cat1* and *Cat2* Transcripts in the Presence of Exogenous ABA. Embryos isolated from 20 dpp kernels of the normal inbred line W64A were incubated for 2 days in the dark on GM supplemented with various levels of ABA. *Cat1* transcript accumulation, like that of the ABA-regulated E_m transcript (Fig. 3), increased with increasing levels of ABA. Conversely, *Cat2* transcript was undetectable at exogenous ABA concentrations above 10^{-6} M.

To determine the time course of induction of *Cat1* transcript accumulation in the presence of ABA, excised 20-dpp

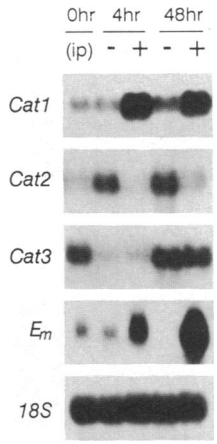

FIG. 2. Differential accumulation of the *Cat* transcripts in the presence and absence of exogenous ABA. Embryos were excised from kernels of the inbred maize line W64A at 18–21 dpp and incubated for 4 and 48 hr in the dark on GM alone (−) and GM supplemented with 10^{-4} M racemic (*R*, *S*)-ABA (+). Total RNA was isolated from treated embryos as well as embryos isolated from kernels 18–21 dpp [*in planta* (ip) or 0 hr], and the accumulation of the various *Cat* transcripts in each sample was determined by Northern blot analysis. Filters were then stripped and reprobed with insert DNA containing the coding sequence of the ABA-regulated E_m polypeptide and subsequently with a DNA fragment containing an 18S ribosomal sequence to ensure similar loading and transfer.

embryos from W64A were incubated in the dark on GM supplemented with 10^{-4} M racemic ABA. Embryos were harvested at intervals over a 2-day period beginning 1 hr after the start of the incubation period. Steady-state levels of *Cat1* and *Cat2* transcript were determined at each time point by Northern blot analysis of total RNA. Accumulation of the *Cat1* transcript had increased significantly by 2 hr (Fig. 3*B*), similar to the accumulation profile of the ABA-regulated E_m message. This strongly suggests that up-regulation of *Cat1*, like E_m, is a direct response to ABA. In contrast, levels of

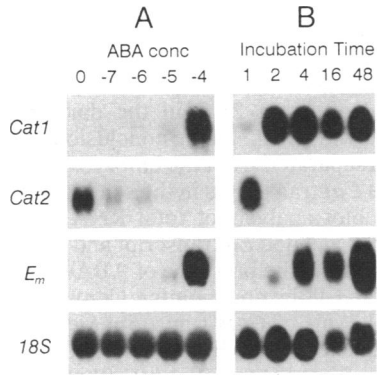

FIG. 3. Dose–response and kinetics of accumulation of *Cat1* and *Cat2* transcripts in the presence of exogenous ABA. Embryos were dissected from developing kernels of the maize inbred line W64A at 20 dpp. (*A*) Excised embryos were incubated for 48 hr in the dark on GM alone (0) or GM supplemented with 10^{-7} M–10^{-4} M racemic (*R*, *S*)-ABA (−7 through −4). (*B*) A second group of excised embryos was incubated in the dark on GM supplemented with 10^{-4} M racemic (*R*, *S*)-ABA and harvested at the indicated intervals (1–48 hr) over a 2-day period starting 1 hr after the beginning incubation. Total scutellar RNA was extracted from each treatment, and steady-state levels of the *Cat1* and *Cat2* transcript were determined by Northern blot analysis. Filters were then stripped and reprobed with insert DNA containing the coding sequence of the ABA-regulated E_m polypeptide and subsequently with a DNA fragment containing an 18S ribosomal sequence to ensure similar loading and transfer.

Cat2 transcript were initially low and had declined below detection by 2–4 hr.

Accumulation of *Cat1* Transcript in Scutella of Developing Embryos of Two Viviparous Mutants and Their Wild-Type Siblings. In addition to analyzing the response to exogenous application of ABA, we used maize viviparous (*vp*) mutant lines to clarify the possible role of ABA in the observed differential CAT response. The maize *vp* mutant lines utilized make either lowered amounts of ABA (line M1A4; *vp5*) or are phenotypically insensitive to normal endogenous levels of ABA (line 326B; *vp1*) (9, 10). In the first set of studies, ears containing both mutant (*vp5/vp5*) and wild-type (*Vp5/—*) kernels were harvested from heterozygous plants of the ABA-deficient viviparous line M1A4 (*Vp5/vp5*). Total RNA was extracted from scutella isolated from mutant kernels and their wild-type siblings at 18, 21, 24, and 27 dpp. Accumulation of *Cat1*, *Cat2*, and E_m transcripts at each developmental time point was analyzed by Northern blot analysis. The level of ABA (pmol of ABA per assay) was also determined for each time point by using tracer competition ELISA (IDETEK). The embryos of *vp5* mutant kernels (*vp5/vp5*) accumulated lower steady-state levels of the *Cat1* RNA and the ABA-regulated E_m transcript (Fig. 4*A*); this lower transcript accumulation closely corresponded to lower endogenous ABA levels (<20% of the comparably aged wild-type sibling embryos; *Vp5/—*) (Fig. 4*B*). The *Cat2* transcript was not detected in line M1A4. Two developmental time points (21 and 27 dpp) were examined for the *vp1* mutant. Accumulation of *Cat1* transcript, as well as ABA at corresponding time points, was essentially identical in scutella of both mutant (*vp1/vp1*) and wild-type (*Vp1/—*) embryos (Fig. 4). In contrast to the pattern of *Cat1*, accumulation of the *Vp1*/ABA-regulated E_m transcript was greatly reduced in the scutella of mutant vs. wild-type sibling embryos.

Accumulation of *Cat1* Transcript in Scutella of Excised Embryos of Viviparous Mutants and Their Wild-Type Siblings in Response to Exogenous ABA. Changes in the accumulation of *Cat1*, *Cat2*, and E_m transcripts in the presence of exogenously applied ABA in both *vp1* and *vp5* were also examined (Fig. 5). Ears containing both mutant (*vp/vp*) and wild-type (*Vp/—*) kernels were harvested from heterozygous plants of the ABA-deficient viviparous line M1A4 (*Vp5/vp5*) and the ABA-insensitive line 368B (*Vp1/vp1*) at 21 and 27 dpp. Mutant and wild-type embryos were excised and incubated for 48 hr at 25°C in the dark on GM or GM supplemented with ABA. At the conclusion of the treatment, scutella were separated from the embryo axes, and levels of the various *Cat* transcripts in the scutella were determined by Northern blot analysis of total extracted RNA. As expected, in the presence of exogenous ABA, both *Cat1* and E_m transcripts accumulated to higher levels in the scutella of both mutant and wild-type sibling embryos of the ABA-deficient line M1A4 (*vp5*). The *Cat2* transcript was not detected in M1A4. In contrast, whereas both *Cat1* and E_m were strongly up-regulated by exogenous ABA in the scutella of wild-type sibling embryos of the ABA-insensitive *vp1* mutant, only *Cat1* was up-regulated normally by exogenous application of ABA in scutella of the mutant embryos. Reduction of *Cat2* accumulation in the *vp1* mutant line in the presence of exogenous ABA was the same in both mutant and wild type.

DISCUSSION

When immature embryos are excised from developing maize seed at ≈21 dpp and placed on hormone-free GM, they germinate precociously. When placed on medium supplemented with ABA, however, such embryos do not germinate, but continue to mature. Suppression of precocious germination and promotion of normal embryo maturation/embryogenesis by ABA are further demonstrated by the observation

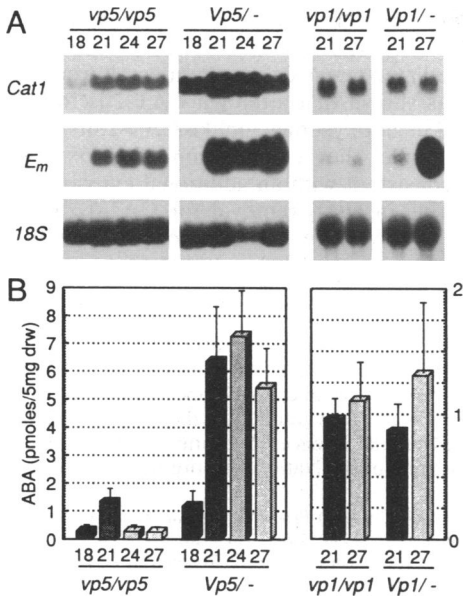

FIG. 4. Changes in *Cat1* transcript accumulation and endogenous ABA levels in scutella of developing embryos of two maize viviparous mutants. Ears containing both mutant (*vp/vp*) and wild-type (*Vp/—*) kernels were harvested from heterozygous plants of both the ABA-deficient viviparous line M1A4 (*vp5*) and the ABA-insensitive line 326B (*vp1*). For *vp5*, scutella of both mutant embryos (*vp5/vp5*) and their wild-type siblings (*Vp5/—*) at 18, 21, 24, and 27 dpp were analyzed. For *vp1*, two developmental time points (21 and 27 dpp) were examined. (*A*) Accumulation of *Cat1* and E_m transcripts at each developmental time point was analyzed by Northern blot analysis of total RNA. Filters were then stripped and reprobed with an 18S ribosomal sequence to ensure similar loading and transfer. Representative analyses are shown. (*B*) Relative levels of active (*R*)-ABA (pmol of ABA per assay) were determined for each developmental time point using trace competition ELISA (IDETEK). ABA was extracted from 50 mg of lyophilized scutellar tissue for each developmental time point, resulting in pooled samples of from 3 to 10 scutella each. Each assay contained extract from the equivalent of 5 mg (dry weight) of scutella. The mean and range for four repetitions are shown.

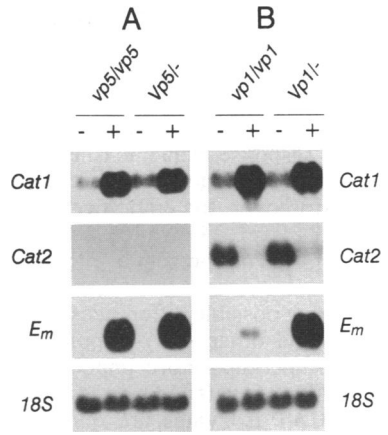

FIG. 5. Accumulation of *Cat1* and *Cat2* transcripts in response to exogenous ABA in scutella of excised embryos of two maize viviparous mutants. Ears containing both mutant (*vp/vp*) and wild-type (*Vp/—*) sibling kernels were harvested from heterozygous plants of both the ABA-deficient viviparous line M1A4 (*vp5*) (*A*) and the ABA-insensitive line 326B (*vp1*) (*B*) at 21 dpp. Excised embryos from both mutant and wild-type kernels were incubated for 48 hr in the dark on GM alone (−) or GM supplemented with 10^{-4} M racemic (*R, S*)-ABA (+). Total RNA was extracted from scutella of treated embryos and accumulation of *Cat1*, *Cat2*, and E_m transcripts was analyzed by Northern blot analysis. Filters were then stripped and reprobed with an 18S ribosomal sequence to ensure similar loading and transfer. The *Cat2* transcript was not detected in the scutella of M1A4 (*vp5*). Representative analyses are shown.

that viviparous mutants of maize, in which embryos germinate precociously in the developing seed, are either lacking in endogenous ABA or are insensitive (at least in this aspect) to the effects of ABA (9–11, 23). This ABA-mediated branch point in development involves the differential regulation of embryogenic and germination-specific gene sets, along with associated metabolic changes. Our results show that at least two of the CATs, important components of the plant's antioxidant defenses, are differentially regulated by ABA during this significant developmental shift.

Using excised, developing maize embryos, we have demonstrated that each of the three CATs responds differently to exogenously applied ABA (Figs. 1–3). Accumulation of *Cat1* transcript and protein increased in the presence of exogenously applied ABA, with *Cat1* RNA accumulating to high levels within 2 hr (Fig. 3). In contrast, steady-state levels of *Cat2* RNA and CAT-2 protein decreased, whereas *Cat3* transcript and CAT-3 protein levels were not affected. Maize viviparous mutants that make either reduced amounts of ABA (*vp5*) or are phenotypically insensitive to normal endogenous levels of ABA (*vp1*) were employed to verify the involvement of ABA in this response. Developmental studies with *vp5* mutant plants showed that both *Cat1* and E_m RNA accumulation was noticeably reduced in the scutella of mutant embryos (compared to levels in wild-type siblings of the same developmental stage). These lower steady-state RNA levels correlated with lower endogenous ABA levels as measured by competitive ELISA (20). In contrast, in *vp1* (ABA-insensitive) mutant plants, both *Cat1* and *Cat2* transcript levels and measured ABA levels were comparable in scutella of both mutant and wild-type siblings, whereas E_m RNA accumulation was much reduced in the mutant. These results are consistent with previous observations of CAT isozyme accumulation in the scutella of developing embryos of the CAT-normal maize inbred line W64A (5). In those studies, CAT-1 protein and *Cat1* transcript accumulated throughout mid to late embryo development, with transcript levels decreasing during the final stages of development. CAT-2 protein and *Cat2* RNA were not detected until the later stages of embryogenesis. Together with our results, this suggests that, although the *Cat2* gene is apparently capable of being expressed by the middle stages of embryo formation (≈20 dpp), the high levels of *Cat2* expression seen during germination (and precocious germination) may be suppressed by ABA during normal embryogenesis *in planta*. This may be analogous to the reported repression of *RbcS* gene expression by ABA during mid to late embryogenesis in wheat (24). The failure to detect the *Cat2* transcript in either viviparous or wild-type sibling embryos of line M1A4 was found to be the result of a *Cat2* null mutation in this line. This mutation did not alter the pattern of *Cat1* or *Cat3* transcript accumulation relative to those seen in the normal maize line W64A (unpublished results).

Not surprisingly, the accumulation of both *Cat1* and E_m transcripts increased in the presence of exogenously applied ABA in both mutant and wild-type embryos of the ABA-deficient mutant *vp5*. In contrast, while *Cat1* levels increased (and *Cat2* levels decreased) in the presence of exogenous ABA in scutella of both mutant and wild-type embryos of the ABA-insensitive *vp1* mutant, normal up-regulation of the ABA-regulated E_m transcript was observed in only wild-type embryos. This suggests that, in contrast to the *Vp1*-mediated regulation of ABA-responsive genes reported to date (11, 23, 25–27), the *Vp1* gene product might not be essential for the ABA-mediated regulation of *Cat1* expression. The down-regulation of *Cat2* in the presence of ABA, like that of *RbcS*,

is probably not linked directly with *Vp1*. Recent sequence and primer extension analyses of *Cat* genomic clones (L. Guan and J.G.S., unpublished results; M. Abler and J.G.S., unpublished results) are consistent with this interpretation. The 5′ upstream region of the *Cat1* gene was found to contain one or more ABA response elements (the *Em1a* consensus; ref. 28), whereas this element was not present in the *Cat3* 5′ genomic region. Moreover, the embryogenic regulatory *Sph* box (5′-CATGCAT-3′; ref. 29), while present in the 5′ region of the E_m gene (30), was not present in either *Cat1* or *Cat3* 5′ genomic regions.

Besides elucidating possible differences between the ABA response of *Cat1* and other ABA-responsive genes, these results have other, perhaps more important, implications. While all three CATs convert H_2O_2 to less toxic molecules, the three maize CAT isozymes are quite distinct. It has previously been shown that the individual maize CATs exhibit different patterns of developmental and tissue-specific expression, as well as differential response to environmental signals such as light, temperature, fungal toxins, etc. (2, 31, 32). This differential response implies that promoter regions of each *Cat* gene contain multiple control elements (the number and combinations varying in each), resulting in a complex, multilayered pattern of response unique to each CAT. For example, preliminary results show that steady-state levels of *Cat1* transcript increase slowly over the first 10 days postimbibition in the scutella of germinating embryos; this is in a tissue where measured levels of ABA are low and decreasing (refs. 17 and 31; A. Acevedo and J.G.S., unpublished results). This is reflected in Fig. 2, where, starting from a relatively high basal level of RNA, the *Cat1* RNA level increases slowly over the time course of the experiment in the absence of ABA (precocious germination). In the presence of ABA, the increased levels of *Cat1* RNA accumulation elicited by the ABA itself are superimposed on top of this non-ABA-mediated change in expression.

In addition to displaying disparate patterns of expression, the CATs are biochemically distinct. CAT can catalyze either the direct dismutation of H_2O_2 into H_2O and O_2 (the catalytic mode) or use H_2O_2 to oxidize substrates such as methanol, ethanol, formaldehyde, formate, and nitrite (the peroxidatic mode) (33). In maize bundle-sheath cells, the peroxisomal CAT (CAT-2) has high catalatic but low peroxidatic activity, whereas CAT-3, which is expressed in leaf mesophyll, has high peroxidatic but low catalytic activity (34, 35). The CAT isozymes also exhibit varying degrees of sensitivity to inhibitors (2). This suggests that each CAT has a specific and distinct metabolic role.

While biochemically distinct, the three CAT isozymes are similar enough structurally to interact *in vitro* to form heterotetramers (1). Only CAT-1 and CAT-2 isozymes, however, form the expected heterotetramers *in vivo* (34), even where the expression of all three CAT isozymes coincides (3). This implies that, although all are encoded by nuclear genes, the CATs are differentially compartmentalized in the cell. Cell fractionation studies verify that both CAT-1 and CAT-2 isozymes are cytosolic or, where glyoxysomes/peroxisomes are present, peroxisomal, whereas CAT-3 coisolates with the mitochondrial cell fraction (3).

It is reasonable to hypothesize that, if biochemically distinct forms of an enzyme are localized in specific subcellular compartments in specific cell types, the function of each isozyme is linked to the metabolic processes occurring in that compartment. We propose that the differential responses of the three CATs to ABA reflect specific functions in dealing with developmentally modulated, metabolic production of H_2O_2. Because ABA also appears to mediate a number of plant responses to environmental stresses such as wounding, salt stress, and desiccation (21, 36, 37), perhaps CAT-1 has, among other functions, a primary role in dealing with H_2O_2 produced in specific cellular/subcellular locations as a result of ABA-mediated stress or developmental processes. Previous work as shown that although CAT-1 is found together with CAT-2 in glyoxysomes, unlike CAT-2, its accumulation does not parallel the accumulation of glyoxysomes. This would be consistent with an additional cytosolic or nonglyoxysomal role in H_2O_2 removal for CAT-1. Similarly, the repression of CAT-2 by ABA is consistent with a role in dealing primarily with glyoxysomal H_2O_2 (e.g., produced by β-oxidation during germination).

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